REMARKS

Claims 2-4, 6-7, 10, and 16-21 are pending. Claims 5, 8-9, 11, and 13-15 have been cancelled without prejudice or disclaimer. Support for new claim 16 is found in the specification at page 10, lines 10-14. Support for new claim 17 is found in the specification at page 8, lines 3-10. Support for new claims 18-21 is found in the specification at page 8, line 11 through page 9 line 11. As disclosed in the specification at page 9, lines 1-8, bipyridine and phenanthroline include substituted derivatives.

The specification and claim amendments are presented in a revised format per the USPTO's announcement 'Amendments in a Revised Format Now Permitted', signed 31 January 2002, and accordingly do not conform to the current reading of 37 C.F.R. §1.121, which Applicants understand has been waived. Accordingly, a complete listing of all claims that are, or were in the application, along with an appropriate status identifier, is provided above in the section entitled "Amendments to the Claims". Markings are provided on replacement paragraphs and claims amended in the present amendment.

Applicants thank the Examiner for granting the highly informative interview that took place on October 17, 2002.

As a preliminary matter, the disclosure is objected to because Structure 3 and Structure 4 are incomplete. Structures 3 and 4 have been amended. Applicants respectfully request withdrawal of the objection.

The Invention:

As discussed during the interview, the present invention is directed to methods of making modified nucleosides comprising electron transfer moieties (ETMs) that can be used in electrochemical detection systems. These electrochemical detection systems allow the determination of the presence of double stranded nucleic acids (e.g. when a target nucleic acid is present) for use in a variety of applications, including diagnostic testing for the presence of pathogenic organisms, particular SNPs, etc. The detection system relies on the

use of capture probes attached to an electrode and at least one ETM present in the hybridization complex. The ETM is provided by a modified nucleoside comprising a covalently attached ETM. Electron transfer between the ETM and the electrode allows for detection. A variety of configurations of the system can be used, but they all rely on the fact that in the absence of a target sequence an electronic signal is not generated. Electronic devices can be used to detect a target sequence of interest, such as sequences from a variety of bacterial and viral pathogens.

Components of this electrochemical system include nucleosides modified at the 2' position with electron transfer moieties (see Exhibit 2). The present invention describes a general synthetic scheme comprising reacting an anhydro-nucleoside with a primary amine or an electron transfer moiety with a primary amine in the presence of an activation agent (see Exhibits 3 and 4). This general synthetic scheme can be used to: 1) add polydentate ligands followed by the addition of a metal ion; 2) add a transition metal chelated by one or more polydentate ligands; and 3) add electron transfer moieties such as ferrocene.

Since the filing of this application, the general synthetic scheme disclosed for the synthesis of modified nucleosides has been used to synthesize a vast array of modified nucleosides. See for example, Krider, et al. (2002) Bioconjugate Chem., 13:155-162 (attached as Exhibit 5); Krider, et. al. (2001) Inorg. Chem., 40:4002-4009 (attached as Exhibit 6); Rack, et al. (2002) J. Am. Chem. Soc., 122:6287 (attached as Exhibit 7); and, Frank and Meade, (2003) Inorganic Chem., 42:1039-1044 (attached as Exhibit 8).

Rejections under 35 U.S.C. § 112, second paragraph

Claims 2-11 and 13-14 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite.

Claims 7, 2 and 3 are rejected for using the term "comprising". Claims 7, 2 and 3 have been amended. Applicants respectfully request withdrawal of the rejection.

The Examiner has reiterated his rejection of Claim 7 for use of the terms "cyclization agent" and "cyclized intermediate".

To reiterate section 2173.05(a) of the M.P.E.P., "the meaning of every term used in a claim should be apparent from the prior art or from the specification and drawings at the time the application is filed".

As argued previously, "cyclization agent" and "cyclized intermediate" are art recognized terms. For example, McGee, et al., (1996) Tetrahedron Letters, 37:1995-1998, describe methods for preparing cyclized intermediates. *See* page 1996 describing the treatment of 2,2'-anhydrouridine intermediates with DBU to effect cyclization to 2'-deoxy-2'-alkoxyamino uridine derivates **9**, **11** and **13** (attached as Exhibit 9). Sebesta, et al. ((1996) Tetrahedron, 52:14385-14402) on page 14387 describe the use of DBU to effect the cyclization of anhydrouridine derivatives (attached as Exhibit 10). McGee, et al. ((1996) J. Org. Chem., 61:781-785) at page 780 describes thermal cyclization of anhydrouridine to obtain the cyclized intermediate **4a** shown in Scheme 1 (attached as Exhibit 11). Ferris and Yanagawa ((1984) J. Org. Chem., 49:2121-2125) describe the use of diiminosuccinonitrile (DISN) and BrCN to effect the cyclization of 3'-nucleotides to the corresponding 2',3'-cyclic nucleotides (attached as Exhibit 12).

The specification at page 20., lines 12-17 defines "cyclization agent" as:

> an agent such as a weak base that breaks the 2,2'- and 2,3'- or 2,5' oxygen bridge between the ribose and the base and forms a ring structure including the 2' and 3' positions of the ribose, such that at either the 2' or 3' position a nitrogen atom is directly attached.

Moreover, examples of a cyclization agent and cyclized intermediates are provided in the specification. In Figure 2, reaction e) demonstrates the formation of a cyclized intermediate (e.g. 5'-O-(4,4'-dimethoxytrityl)-2'-N,3'-O-(2-oxooxazolidinyl)-2'-aminomethylpyridyl-2'-deoxyruidine) in the presence of the cyclization agent 1,8-

diazabicyclo-undec-7-ene (DBU). At page 20, line 17, provides 1,4-diazabicyclo-octane (DBO) as another example of a cyclization agent.

In addition, Applicants submit the declaration by Dr. Thomas Meade. As can be seen from Dr. Meade's curriculum vitae (attached as Exhibit 1), Dr. Meade has extensive experience in designing and synthesizing modified nucleosides.

As set forth in paragraph 5, Dr. Meade states that it is his belief that the term "cyclization agent" would be understood to refer to a catalytic agent, such as DBU, DISN, or BrCN, that can effect the cyclization of anyhydronucleosides.

As set forth in paragraph 6, Dr. Meade states it is his belief that the term "cyclized intermediate" would be understood to refer to bicyclic nucleoside derivatives such as 2'-deoxy-2'-alkoxyamino uridine derivatives, oxazoline derivatives, and 2',3'-cyclic nucleotides.

As can be seen from the above discussion, a person of ordinary skill in the art would know what is meant by the terms "cyclization agent" and "cyclized intermediate" as used in the context of the specification in connection with the synthesis of modified nucleosides. Applicants respectfully request withdrawal of the rejection of Claim 7 under 35 U.S.C. § 112, second paragraph.

The Examiner has reiterated his rejection of Claims 2 and 3, for lacking steps involved in the process of adding phosphoramidite groups and phosphoramidite-derivatized nucleosides to the terminus of a growing oligonucleotides chain.

Applicants assert that a person of ordinary skill in the art would know the steps involved in the process of adding phosphoramidite groups and phosphoramidite-derivatized nucleosides to the terminus of a growing oligonucleotides chain.

Applicants present several articles in support of their position that the process of adding phosphoramidite groups and phosphoramidite-derivatized nucleosides to the terminus of a growing oligonucleotides chain would be well known to a person of ordinary skill in the

art. The applicants are not using subsequent work to supplement the disclosure of the application; rather, the subsequent work is presented to show that the utility asserted and shown in the application is supported by further research, and that the specification fully enables the synthesis of metal containing nucleosides. See In re Wilson, 135 USPQ 442, 444 (CCPA 1962); Ex parte Obukowicz, 27 USPQ 2d 1063 (BPAI 1993); Gould v. Quigg, 3 USPQ 2d 1302,1305 (Fed. Cir. 1987):

> "it is true that a later dated publication cannot supplement an insufficient disclosure in a prior dated application to render it enabling. In this case the later dated publication was not offered as evidence for this purpose. Rather, it was offered . . . as evidence that the disclosed device would have been operative" printed publications.

Articles by Meade and Kayyem (1995) Angew. Chem. Int. Ed. Engl. 34:352-353 (attached as Exhibit 13), Yu et al. (2001) J. Org. Chem., 66:2937-2942 (attached as Exhibit 14); Krider, et al. (2001) Inorg. Chem., 40:4002-4009 (attached as Exhibit 6); Krider et al. (2002) Bioconjugate Chem., 13:155-162 (attached as Exhibit 5); Frank and Meade (2003) Inorg. Chem. 42:1039-1044 (attached as Exhibit 8) and Anne, et al. (2001) Bioconjugate Chem., 12:396-405 (attached as Exhibit 15), describe the addition of phosphoramidite groups to modified nucleosides labeled with bulky substitutents, such as fluorophores, transition metal complexes, etc., at the 2' position of ribose for incorporation into nucleic acids both enzymatically and chemically.

Applicants note that none of above references provide a detailed description for the addition of a phosphoramidite moiety to a nucleoside or incorporation of the phosphoramidite modified nucleoside into an oligonucleotide. Instead, the above references refer the reader to established procedures for the preparation of DMT protected nucleoside phosphoramidites and subsequent oligonucleotide synthesis. For example, Meade and Kayyem state that a DMT-2'-N-trifluoracetyl-protected phosphoramidite of 2'-amino-2'-deoxyuridine was prepared by variation of published procedures and reference Yamaguchi and Hirao (1983) 24:391 and that oligodeoxyribonucleotides were assembled by standard solid phase

automated DNA synthesis techniques referencing Kline, et al. (1990) J. Am. Chem. Soc., 112:7373; Nibonowicz and Pardi (1992) Nature, 355:184; Batey, et al., (1992) Nucleic Acids Res., 20:4515; Michnicka, et al. (1993) Biochemistry, 32:395; Quant, et al. (1994) Tetrahedron Lett., 35:6649 (see Exhibit 13).

The specification at page 21, line 1-22 also outlines general methods for converting a modified nucleoside into the phosphoramidite form:

> Alternatively, and preferably, the amino nucleoside is converted to the phosphoramidite or H-phosphonate form, which are then used in solid-phase or solution syntheses of oligonucleotides. In this way the modified nucleoside is incorporated into the oligonucleotide at either an internal position or a terminus. This is generally done in one of two ways. First, the 5' position of the ribose is protected with 4',4-dimethoxytrityl (DMT) followed by reaction with either 2-cyanoethoxy-bis-diisopropylaminophosphine in the presence of diisopropylammonium tetrazolide, or by reaction with chlorodiisopropylamino 2'-cyanoethyoxyphosphine, to give the phosphoramidite as is known in the art; although other techniques may be used as will be appreciated by those in the art. See Gait, supra; Caruthers, Science 230:281 (1985), both of which are expressly incorporated herein by reference.

Finally, as set forth in paragraph 8, Dr. Meade states that oligonucleotide synthesis using phosphoramidite chemistry involves a series of deprotection, coupling, capping, and oxidation steps that are repeated until the specified nucleotide chain is constructed. The details of which are set forth in standard reference books such as Gait, M., ed. (1984) *Oligonucleotide Synthesis: A Practical Approach*, Oxford University Press, Oxford, and Eckstein, F., ed. (1991) *Oligonucleotides and Analogues: A Practical Approach*, Oxford University Press, Oxford.

As can be seen from the above discussion, a person of ordinary skill in the art would know the steps required for adding a phosphoramidite group to a modified nucleoside and for adding a phosphoramidite-derivatized nucleoside into a growing oligonucleotide chain. Applicants respectfully request withdrawal of the rejection of Claims 2 and 3 under 35 U.S.C. § 112, second paragraph.

The Examiner has reiterated his rejection of Claim 5 for use of the term "nucleoside analog". Claim 5 has been cancelled without prejudice or disclaimer and thus the rejection is moot.

Claim 8 is rejected because the term "electron transfer moiety is a transition metal complex comprising a transition metal and at least one ligand" is technically incorrect because it refers to both compounds and substituents. Claim 8 has been cancelled and thus the rejection is moot. Applicants note that new claim 16 refers to an electron transfer moiety that is ferrocene. Applicants submit that the use of the term "electron transfer moiety" is not inconsistent with the term "ferrocene" as ferrocene is capable of electron transfer and may be used to detect nucleic acids. See for example, the specification at page 7, lines 13-18 and Exhibit 14.

Claim 9 is rejected because the term transition metal complex comprises a transition metal is technically incorrect and incomplete. Claim 9 has been cancelled and thus the rejection is moot.

Claim 10 is rejected for use of the term "polydentate ligand". For the sake of clarity, Claim 10 has been amended to recite a method for making a modified nucleoside comprising at least one covalently attached polydentate ligand that chelates a transition metal.

The specification beginning at page 8, line 11 states that transition metals can be chelated by at least one polydentate ligand that is covalently attached to the nucleoside. The ligand(s) provide the coordination atoms for the binding of the metal ion. The number and nature of the ligands will depend on the coordination number of the metal ion. Thus, a polydentate ligand is a ligand that is attached to a central metal ion by bonds from two or more donor atoms. Depending on the ligand, donor atoms may be preferably oxygen, nitrogen or phosphorus (see page 9, lines 12-15). Suitable ligands for the metal ions disclosed in the specification are outlined beginning at page 8, line19, through page 9, line 11.

As argued previously, "polydentate ligand" is an art recognized term. For example, Bianchini, et al. (1990) J. Am. Chem. Soc., 122:9190-9197 (attached as Exhibit 18); Klink et al. (2002) J. Phys. Chem., 106:3681-3689 (attached as Exhibit 19); and Browne, et al. (2001) J. Chem. Soc., Dalton Trans., 2001:3513-3519 (attached as Exhibit 20) use the term polydentate ligand without further definition. Moreover, these Exhibits illustrate that the number and nature of the ligand depends on the metal ion. For example, Browne et al. (Exhibit 20) provides an example of a cobalt(III) complex in which the polydentate ligand is attached to the cobalt ion by bonds from five donor nitrogen atoms and one donor halogen atom. Similarly, Bianchini et al. (Exhibit 18) describes P_2N_2 ligands that are bidentate through the phosphorous donor atoms, tridentate through the two phosphorous and a central nitrogen donor atoms and tetradentate when all of the phosphorous and nitrogen donor atoms coordinate to a metal.

As stated above, applicants are not using subsequent work to supplement the disclosure of the application; rather, the subsequent work is presented to show that the utility asserted and shown in the application is supported by further research, and that the specification fully enables the synthesis of metal containing nucleosides. See In re Wilson, *supra*; Ex parte Obukowicz, *supra*; and, Gould v. Quigg, *supra*.

In addition, as set forth in paragraph 10, Dr. Meade states that it is his belief that the term "polydentate ligand" would be understood to refer to a ligand that is attached to a central metal ion by bonds from two or more donor atoms. Ligands with suitable donor atoms include ligands such as P_2N_2, bipyridine, and the like that use nitrogen, oxygen, phosphorus, and halogen atoms.

As can be seen from the above discussion, a person of ordinary skill in the art would know what is meant by the term "polydentate ligand". Applicants respectfully request withdrawal of the rejection of Claim 10 under 35 U.S.C. § 112, second paragraph.

Claims 11-14 are rejected for not providing a description of the structure being claimed. Claims 11-14 have been cancelled and thus the rejection is moot.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 2-11 and 13-15 are rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. Specifically, claims 7 and 10 are rejected for the use of the generic term "anydro-nucleoside". Applicants respectfully submit that the term "anydro-nucleoside" is not generic.

As argued previously, "anydro-nucleoside" is an art recognized term. For example, Ferris and Yanagawa (1984) J. Org. Chem., 49:2121-2125 (attached as Exhibit 12); David and de Sennyey (1982) J. Chem. Soc., Perkin Trans. 1, 2:385-93 5 (attached as Exhibit 16); Qiu, et al., (1998) Angewandte Chemie, International Edition, 37:1440-415 (attached as Exhibit 17); Mizuno and Sasaki (1965) Tetrahedron Lett., 50:4579-4584 (attached as Exhibit 21); Robins and Kanai (1976) J. Org. Chem., 41: 1886-1887 (attached as Exhibit 22); and Kaneko et al. (1978) Nucleic Acid Chemistry, 595-599 (attached as Exhibit 23); and Jung, et al. (1998) Nucleosides & Nucleotides, 17:2383-2387 (attached as Exhibit 24) use the term "anyhdro-nucleoside" without further definition.

Moreover, definitions for "anydronucleoside" and "activated anhydronucleoside" are provided in the specification. For example, "anydronucleoside" is defined as a "2,2'-, 2,3'- or 2,5' anydronucleoside, comprising an oxygen bridge between the C-2 of the base pyrimidine and the C-2' or C-3' of the ribose or ribose analog." See specification at page 18, line 23 through page 19, line 13. An "activated anhydronucleoside" is formed when an anhydronucleoside and a signalling moiety comprising a primary amine are added together in the presence of an activation agent. See page 20, lines 1-2. On page 20, lines 10-11, an "activated anhydronucleoside" is defined as an "anhydronucleoside ready to react with the signalling moiety comprising a primary amine to form a carbamate."

As set forth in paragraph 11, Dr. Meade states it is his belief that the term "anydronucleoside" refers to pyrimidine nucleosides comprising an oxygen bridge between

the C-2 of the base pyrimidine and C-2' or C-3' of the ribose or purine nuclosides that have an oxygen bridge between the C-8 of the purine residue and a hydroxyl group, e.g., C-2' or C-3' of the ribose.

As can be seen from the above discussion, a person of ordinary skill in the art would know what is meant by the term "anhydronucleoside". Applicants respectfully request withdrawal of the rejection of Claims 7 and 10 under 35 U.S.C. § 112, first paragraph.

The Examiner states that the description lacks confirmatory spectra. Applicants respectfully submit that there is no requirement for providing confirmatory spectra under 35 U.S.C. § 112, first paragraph. As set forth in MPEP 2163:.

> An applicant shows possession of the clamed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention.

As the specification includes structures, figures, diagrams, and formulas for making 2' modified nucleosides, Applicants submit that there is sufficient written description to clearly convey Applicants invention.

The paragraphs appearing at pages 26-27 have been amended to refer to the chemical products appearing in Figure 2. Additionally, a third paragraph has been added to identify the structures in Figure 3. The latter description has been added based on the Examiner's assurance that such a description would not constitute new matter. Finally, the specification has been amended to provide definitions for the acromyms DMTCl, DIEA, DBU, and to correctly refer to the solvent dichloromethane CH_3Cl_2.

Rejections under 35 U.S.C. § 103(a)

Claims 2-11 and 13-15 are rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 95/35102 (referred to as Nexstar).

The Nexstar reference discloses methods for making labeled nucleosides from anyhdro-nucleosides containing metal alkoxides. See page7, line 25, through page 9, line 20. The resulting modified nucleosides are used as drugs.

In contrast, the present invention is directed to making modified nucleosides comprising an electron transfer moiety and at least one polydentate ligand. The resulting modified nucleosides find use in electrochemical detection systems.

To establish a *prima facie* case of obviousness the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) M.P.E.P. §2143.

There is no teaching in the Nexstar reference of modified nucleosides comprising an electron transfer moiety and at least one polydentate ligand. Therefore, the requirement of teaching or suggesting all the claim elements has not been met. Applicant respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

The Examiner is invited to contact the undersigned at (415) 781-1989 if any issues may be resolved in that manner.

Respectfully submitted,
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